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PATENT COOPERATION TREATY

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FROM THE INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT
issued pursuant to PCT Rule 61.1

DATE OF MAILING by the International Preliminary Examining Authority	17.12.91
APPLICANT's or AGENT's FILE REFERENCE	PCT 0172

IDENTIFICATION OF THE INTERNATIONAL APPLICATION

International Application No.	International Filing Date
PCT/NL 90/00130	11/09/1990

Applicant (Name)

RIJKSUNIVERSITEIT TE LEIDEN et al.

NOTIFICATION

The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the International preliminary examination report and its annexes, if any, established on the above-identified international application.

The attention of the applicant is drawn to the reminder contained in Form PCT/IB/332, which he received from the International Bureau, concerning the time limits within which he must perform certain acts before each elected Office.

A copy of the report and its annexes, if any, has this same day also been transmitted to the International Bureau.

THE INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

Name and Mailing Address	Authorized Officer
European Patent Office Erhardtstraße 27 D-8000 München 2	E. Altmann

Europäisches Patentamt
Generaldirektion 2

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PATENT COOPERATION TREATY
INTERNATIONAL PRELIMINARY EXAMINATION REPORT

IDENTIFICATION OF THE INTERNATIONAL APPLICATION	Applicant's or Agent's File Reference: PCT 0172	
International Application No.: PCT/NL 90/ 00130	International Filing Date: 11/09/1990	Date demand submitted: 11/04/1991
Receiving Office: RO/ NL	Priority Date Claimed: 14/09/1989	
Applicant (Name): RIJKSUNIVERSITEIT TE LEIDEN et al.		

BASIS OF REPORT

1. AMENDMENTS AND/OR RECTIFICATIONS [1*] - The amendments and/or rectifications made before this International Preliminary Examining Authority in respect of the claims, the description, and/or drawings in the above-identified International application are annexed to this report.

a) [X] This report has been established on the basis of the following application documents:

- [ ] the application documents as filed
- [X] description, pages 1-18 ..... as originally filed
- description, pages ..... filed with your letter of
- description, pages ..... filed with your letter of
- description, pages ..... filed with your letter of
- [X] claim(s) ..... as originally filed
- claim(s) 1-48 ..... filed with your letter of 19.08.91
- claim(s) ..... filed with your letter of
- claim(s) ..... filed with your letter of
- [X] drawings, sheet/fig. 1 ..... as originally filed
- drawings, sheet/fig. ..... filed with your letter of

b) [ ] The amendments resulted in the cancellation of the following sheets: .....

c) [ ] This report has been established as if the amendments indicated on the extra sheet have not been made, since, for the reasons indicated, they have been considered to go beyond the disclosure as filed.

2. PRIORITY [2]

a) This report has been established as if no priority has been claimed due to the failure to furnish within the prescribed time limit the requested:

- [ ] copy of the earlier application whose priority has been claimed.
- [ ] translation of the earlier application whose priority has been claimed.

b) [ ] This report has been established as if no priority has been claimed due to the fact that the priority claim has been found invalid.

Thus, for the purposes of this report, the International filing date indicated above is considered to be the relevant date.

* Where replacement sheets are annexed to this report, a translation of these replacement sheets must be furnished to the elected Offices within the time limit applicable under PCT Article 39(1).

FURTHER INFORMATION CONTINUED FROM THE FIRST SHEET

BASIS OF REPORT (Continued)

3. UNITY OF INVENTION [3] — The international application does not comply with the requirement of unity of invention.

a. In response to an invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest. Where requested by the applicant, the text of the protest together with the decision taken thereon are annexed to this report.

☐ neither restricted nor paid additional fees.

b. ☐ No invitation has been issued. The opinion of this International Preliminary Examining Authority is that the international application does not comply with the requirement of unity of invention for the following reasons. (specify)

c. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☐ the parts relating to the restricted claims, that is claims Nos. _____

☐ the parts relating to the main invention, that is claims Nos. _____.

4. NON-ESTABLISHMENT OF REPORT ON QUESTIONS OF NOVELTY, INVENTIVE STEP OR INDUSTRIAL APPLICABILITY [4]

The questions of whether the claimed invention appears to be novel, to involve an inventive step or to be industrially applicable have not for the reasons indicated been gone into in respect of:

a. ☐ the entire international application

b. ☐ claims Nos. _____

for the following reasons:

☐ Said international application, or said claims Nos. _____ relate to the following subject matter which does not require an international preliminary examination. (specify)

☐ The description, claims, or drawings (indicate particular elements) or said claims Nos. _____ are so unclear that no meaningful opinion could be formed.

☐ The claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☐ Said claims Nos. _____ are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all.) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

C12N15/35 C12N5/10 C12P21/02 C12N15/86
G01N33/569 A61K39/23 A61K39/295

REASONED STATEMENT AS TO CLAIMS MEETING CRITERIA OF NOVELTY (N), INVENTIVE STEP (IS) AND INDUSTRIAL APPLICABILITY (IA) [6] AND CITATIONS [7] AND EXPLANATIONS [8] SUPPORTING SUCH STATEMENT

CLAIM NUMBER	STATEMENT (CRITERIA)	CITATIONS AND EXPLANATIONS
1-48	N IS IA	Yes see attached sheet

See notes on accompanying sheet

NON-WRITTEN DISCLOSURES [9]

Kind of Non-Written Disclosure	Date of Written Disclosure referring to the Non-Written Disclosure	Date of Non-Written Disclosure

CERTAIN PUBLISHED DOCUMENTS [10]

Application/Patent	Date of Publication	Filing Date	Priority Date (Valid Claim)

CERTAIN DEFECTS IN THE INTERNATIONAL APPLICATION [11]

The following defects in the form or contents of the international application have been noted.

CERTAIN OBSERVATIONS ON THE INTERNATIONAL APPLICATION [12]

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description have been noted.

see attached sheet

CERTIFICATION

Date Demand Submitted	Date of Completion of the International Preliminary Examination Report
11.04.91	17. 12. 91
International Preliminary Examining Authority	**Signature of Authorized Officer**
EPO	

NOTES TO FORM PCT/IPEA/409

These Notes are intended to facilitate the use of the present form. For full information, see the text of the Patent Cooperation Treaty and the texts of the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and the said texts, the latter are applicable. "Article" refers to Articles of the Treaty, "Rule" refers to Rules of the Regulations and "Section" refers to Sections of the Administrative Instructions.

1 "If the claims have been amended, the report shall issue on the claims as amended." **(Rule 70.2 (a))**

"If the International Preliminary Examining Authority considers that any amendment goes beyond the disclosure in the international application as filed, the report shall be established as if such amendment had not been made, and the report shall so indicate. It shall also indicate the reasons why it considers that the amendment goes beyond the said disclosure." **(Rule 70.2 (c))**

"If, before the International Preliminary Examining Authority, amendments have been made, this fact shall be indicated in the report. Where any amendment has resulted in the cancellation of an entire sheet, this fact shall also be specified in the report." **(Rule 70.11)**

"If the claims, the description, or the drawings, were amended before the International Preliminary Examining Authority, each replacement sheet under Rule 66.8(a) shall be annexed to the report. Replacement sheets superseded by later replacement sheets and letters under Rule 66.8(a) shall not be annexed." **(Rule 70.16)**

2 "If, pursuant to Rule 66.7(a) or (b), the report is established as if the priority had not been claimed, the report shall so indicate." **(Rule 70.2(b))**

"If the International Preliminary Examining Authority needs a copy of the application whose priority is claimed in the international application, the International Bureau shall, on request, promptly furnish such copy. If that copy is not furnished to the International Preliminary Examining Authority because the applicant failed to comply with the requirements of Rule 17.1, the international preliminary examination report may be established as if the priority had not been claimed." **(Rule 66.7(a))**

"If the application whose priority is claimed in the international application is in a language other than the language or one of the languages of the International Preliminary Examining Authority, that Authority may invite the applicant to furnish a translation in the said language or one of the said languages within 2 months from the date of the invitation. If the translation is not furnished within that time limit, the international preliminary examination report may be established as if the priority had not been claimed." **(Rule 66.7(b))**

See also **Rule 70.10** in note **10** below.

3 "If the applicant paid additional fees for the international preliminary examination, or if the international application or the international preliminary examination was restricted under Article 34(3), the report shall so indicate. Furthermore, where the international preliminary examination was carried out on restricted claims (Article 34(3)(a)), or on the main invention only (Article 34(3)(c)), the report shall indicate what parts of the international application were and what parts were not the subject of international preliminary examination." **(Rule 70.13)**

Rule 68 entitled "Lack of Unity of Invention (International Preliminary Examination)" reads as follows:

"68.1 No Invitation to Restrict or Pay

Where the International Preliminary Examining Authority finds that the requirement of unity of invention is not complied with and chooses not to invite the applicant to restrict the claims or to pay additional fees, it shall establish the international preliminary examination report, subject to Article 34(4)(b), in respect of the entire international application, but shall indicate, in the said report, that, in its opinion, the requirement of unity of invention is not fulfilled and shall specify the reasons for which the international application is not considered as complying with the requirement of unity of invention."

"68.2 Invitation to Restrict or Pay

Where the International Preliminary Examining Authority finds that the requirement of unity of invention is not complied with and chooses to invite the applicant, at his option, to restrict the claims or to pay additional fees, it shall specify at least one possibility of restriction which, in the opinion of the International Preliminary Examining Authority, would be in compliance with the applicable requirement, and shall specify the amount of the additional fees and the reasons for which the international application is not considered as complying with the requirement of unity of invention. It shall, at the same time, fix a time limit, with regard to the circumstances of the case, for complying with the invitation; such time limit shall not be shorter than 1 month, and it shall not be longer than 2 months, from the date of the invitation."

"68.3 Additional Fees

(a) The amount of the additional fee due for international preliminary examination under Article 34(3)(a) shall be determined by the competent International Preliminary Examining Authority.

(b) The additional fee due for international preliminary examination under Article 34(3)(a) shall be payable direct to the International Preliminary Examining Authority.

(c) Any applicant may pay the additional fee under protest, that is, accompanied by a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive. Such protest shall be examined by a three-member board or other special instance of the International Preliminary Examining Authority, or any competent higher authority, which, to the extent that it finds the protest justified, shall order the total or partial reimbursement to the applicant of the additional fee. On the request of the applicant, the text of both the protest and the decision thereon shall be notified to the elected Offices as an annex to the international preliminary examination report.

(d) The three-member board, special instance or competent higher authority, referred to in paragraph (c), shall not comprise any person who made the decision which is the subject of the protest."

"68.4 Procedure in the Case of Insufficient Restriction of the Claims

If the applicant restricts the claims but not sufficiently to comply with the requirement of unity of invention, the International Preliminary Examining Authority shall proceed as provided in Article 34(3)(c)."

"68.5 Main Invention

In case of doubt which invention is the main invention for the purposes of Article 34(3)(c), the invention first mentioned in the claims shall be considered the main invention."

4 "If the International Preliminary Examining Authority considers

(i) that the international application relates to a subject matter on which the International Preliminary Examining Authority is not required, under the Regulations, to carry out an international preliminary examination, and in the particular case decides not to carry out such examination, or

(ii) that the description, the claims, or the drawings, are so unclear, or the claims are so inadequately supported by the description, that no meaningful opinion can be formed on the novelty, inventive step (non-obviousness), or industrial applicability, of the claimed invention,

the said Authority shall not go into the questions referred to in Article 33(1) and shall inform the applicant of his opinion and the reasons therefor." **(Article 34(4)(a))**

"If any of the situations referred to in subparagraph (a) is found to exist in, or in connection with, certain claims only, the provisions of that subparagraph shall apply only to the said claims." **(Article 34(4)(b))**

"If, at the time of establishing the international preliminary examination report, the International Preliminary Examining Authority considers that any of the situations referred to in Article 34(4)(a) exists, that report shall state this opinion and the reasons therefor..." **(Article 35(3)(a))**

"If a situation under Article 34(4)(b) is found to exist, the international preliminary examination report shall, in relation to the claims in question, contain the statement as provided in subparagraph (a), ..." **(Article 35(3)(b))**

"... Where the national law of the national Office acting as International Preliminary Examining Authority does not allow multiple dependent claims to be drafted in a manner different from that provided for in the second and third sentences of Rule 6.4(a), the International Preliminary Examining Authority may, in case of failure to use that manner of claiming, apply Article 34(4)(b)...." **(Rule 66.2(a))**

Rule 67 entitled "Subject Matter under Article 34(4)(a)(i)" reads as follows:

"67.1 Definition

No International Preliminary Examining Authority shall be required to carry out an international preliminary examination on an international application if, and to the extent to which, its subject matter is any of the following:

(i) scientific and mathematical theories,

(ii) plant or animal varieties or essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes,

(iii) schemes, rules or methods of doing business, performing purely mental acts or playing games,

(iv) methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods,

(v) mere presentations of information,

(vi) computer programs to the extent that the International Preliminary Examining Authority is not equipped to carry out an international preliminary examination concerning such programs."

5 "The report shall repeat the classification given under Rule 43.3 [classification of the subject matter in the international search report] if the International Preliminary Examining Authority agrees with such classification." **(Rule 70.5(a))**

"Otherwise, the International Preliminary Examining Authority shall indicate in the report the classification, at least according to the International Patent Classification, which it considers correct." **(Rule 70.5(b))**

6 "The international preliminary examination report shall not contain any statement on the question whether the claimed invention is or seems to be patentable or unpatentable according to any national law. It shall state, subject to the provisions of paragraph (3), in relation to each claim, whether the claim appears to satisfy the criteria of novelty, inventive step (non-obviousness), and industrial applicability, as defined for the purposes of the international preliminary examination in Article 33 (1) to (4). The statement shall be accompanied by the citation of the documents believed to support the stated conclusion with such explanations as the circumstances of the case may require. The statement shall also be accompanied by such other observations as the Regulations provide for." **(Article 35 (2))**

"The statement referred to in Article 35(2) shall consist of the words "YES" or "NO," or their equivalent in the language of the report, or some appropriate sign provided for in the Administrative Instructions, and shall be accompanied by the citations, explanations and observations, if any, referred to in the last sentence of Article 35 (2)." **(Rule 70.6 (a))**

"If any of the three criteria referred to in Article 35 (2) (that is, novelty, inventive step (non-obviousness), industrial applicability) is not satisfied,

Reasoned statement as to claims meeting criteria of novelty, inventive step and insdustrial applicability.

The subject-matter of all claims is novel, because, due to the difficulties encountered in purifying enough human B19 virus, the VP1 and VP2 proteins have never been isolated and are solely known by their sequences, which may be deduced from the already known sequences of the cloned (but not expressed as such) corresponding genes.

The closest prior art is DocA: Biotechnology 5 (1987), pp.1077-1080: Said document discloses that VP1 may be expressed in an E.coli expression system as a fusion protein with galactosidase. The fused entity is, however, of such a high molecular weight that it is not soluble in the absence of detergent.
No prior art seems to exist in relation to an isolated VP2 protein.
The differences between the prior art concerning VP1 and the present application are as follows:

- native protein is obtained in the latter case.
- because of the expression system used, a higher yield is achieved and the protein structure is closely resembling the human VP1 protein.

In other words, although the VP1 DNA and the expression system were known in the state of the art, their combination led to the successful, efficient production of the parvovirus envelope proteins in a form hitherto not obtained and advantageous for setting up detection assays and immunizing compositions against said virus.
The inventivity and industrial applicability of the claims can, thus be acknowledged (see,however, next page, par.2).)

Objections under Article 6 PCT.

1). Article 6 PCT states that the claims should be clear and concise. This requirement refers to the claims in their entirety as well as to individual claims. Undue repetition of wording between one claim and another should be avoided by the use of the dependent form.
In the present case, 44 out of 48 claims are independent claims which are "linked" to each other by the expression "Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been provided with the genetic information which is necessary for expression of the B19 virus protein..." or variants thereof.
To fulfill the requirements of Art.6 PCT, claims 2-13, 15-27, 29-38 and 40-48 should be tailored down and made dependent on claims 1, 14, 28 and 39 respectively.

2). For the assessment of the present claims 13, 27, 38 and 48 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also depend upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Ln PCT 0172

Re: International Patent Appln.
No. PCT/NL 90/00130

New claims

1. Recombinant non-fused VP1 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of the B19 virus protein VP1.

2. Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been provided with the genetic information which is necessary for expression of VP1 protein of the human parvovirus B19.

3. A method of producing VP1 protein of the human parvovirus B19 by culturing Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been provided with the genetic information which is necessary for expression of the B19 virus protein VP1.

4. A method according to claim 3, wherein the B19 virus protein formed in the cells is isolated from the cells.

5. Recombinant baculovirus expression vector, equipped with the genetic information which is necessary for expression of VP1 protein of the human parvovirus B19 in Spodoptera frugiperda cells.

6. Recombinant baculovirus expression vector pAcB19VP1-YM1.

7. Recombinant baculovirus, equipped with the genetic information which is necessary for expression of VP1 protein of the human parvovirus B19 in Spodoptera frugiperda cells.

8. Recombinant baculovirus AcB19VP1L.

9. The use of recombinant non-fused VP1 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of the B19 virus protein VP1, in an assay for detecting antibodies directed against the B19 virus protein VP1 in a sample to be tested.

10. The use of Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped

with the genetic information that is necessary for expression of VP1 protein of the human parvovirus B19, in an assay for detecting antibodies directed against the B19 virus protein VP1 in a sample to be tested.

11. The use of Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of VP1 protein of the human parvovirus B19, in an IFA or ELISA for detecting antibodies directed against the B19 virus protein VP1 in a sample to be tested.

12. A vaccine preparation for inducing an immune response which provides protection against the human parvovirus B19, comprising recombinant non-fused VP1 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for the expression of the B19 virus protein VP1, or an antigenically active portion of this recombinant B19 virus protein VP1, in combination with one or more carriers and/or adjuvants suitable for vaccination purposes.

13. The use of recombinant non-fused VP1 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of the B19 virus VP1, or with an antigenically active portion of this recombinant B19 virus protein VP1 for inducing an immune response, which provides protection against the human parvovirus B19.

14. Recombinant non-fused VP2 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of the B19 virus protein VP2.

15. Recombinant virus-like particles consisting of VP2 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for the expression of the B19 virus protein VP2.

16. Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of VP2 protein of the human parvovirus B19.

17. A method of producing VP2 protein of the human parvovirus B19, and/or virus-like particles consisting of VP2 protein of the human parvovirus B19, by culturing Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of the B19 virus protein VP2.

18. A method according to claim 17, wherein the B19 virus protein VP2 and/or virus-like particles consisting of VP2 protein of the human parvovirus B19 formed in the cells, are isolated from the cells.

19. Recombinant baculovirus expression vector, equipped with the genetic information that is necessary for expression of VP2 protein of the human parvovirus B19 in Spodoptera frugiperda cells.

20. Recombinant baculovirus expression vector pAcB19VP2-YM1.

21. Recombinant baculovirus, equipped with the genetic information that is necessary for expression of VP2 protein of the human parvovirus B19 in Spodoptera frugiperda cells.

22. Recombinant baculovirus AcB19VP2L.

23. The use of recombinant non-fused VP2 protein of the human parvovirus B19, and/or of virus-like particles consisting of VP2 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression

vector system have been equipped with the genetic information that is necessary for expression of the B19 virus protein VP2, in an assay for detecting antibodies directed against the B19 virus protein VP2 in a sample to be tested.

24. The use of Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information which is necessary for expression of VP2 protein of the human parvovirus B19 in an assay for detecting antibodies directed against the B19 virus protein VP2 in a sample to be tested.

25. The use of Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of VP2 protein of the human parvovirus B19 in an IFA or ELISA for detecting antibodies directed against the B19 virus protein VP2 in a sample to be tested.

26. A vaccine preparation for inducing an immune response which provides protection against the human parvovirus B19, comprising recombinant non-fused VP2 protein of the human parvovirus B19, and/or virus-like particles consisting of VP2 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of the B19 virus protein VP2, or an antigenically active portion of this recombinant B19 virus protein VP2, in combination with one or more carriers and/or adjuvants suitable for vaccination purposes.

27. The use of recombinant non-fused VP2 protein of the human parvovirus B19, and/or of virus-like particles consisting of VP2 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of the B19 virus protein VP2, or with an antigenically active portion of this recombinant B19

virus protein VP2, for inducing an immune response which provides protection against the human parvovirus B19.

28. Recombinant virus-like particles consisting of VP1 and VP2 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of these B19 virus proteins.

29. Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of VP1 and VP2 protein of the human parvovirus B19.

30. A method of producing VP1 and VP2 protein of the human parvovirus B19, and/or virus-like particles consisting of VP1 and VP2 protein of the human parvovirus B19, by culturing Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of these B19 virus proteins.

31. A method according to claim 30, wherein the B19 virus proteins and/or virus-like particles consisting of such proteins, formed in the cells, are isolated from the cells.

32. Recombinant baculovirus expression vector, equipped with the genetic information which is necessary for expression of VP1 and VP2 protein of the human parvovirus B19 in Spodoptera frugiperda cells.

33. Recombinant baculovirus, equipped with the genetic information that is necessary for expression of VP1 and VP2 protein of the human parvovirus B19 in Spodoptera frugiperda cells.

34. The use of recombinant virus-like particles consisting of VP1 and VP2 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for the expression of these B19

virus proteins, in an assay for detecting antibodies directed against the B19 virus in a sample to be tested.

35. The use of Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information which is necessary for expression of VP1 and VP2 protein of the human parvovirus B19, in an assay for detecting antibodies directed against the B19 virus in a sample to be tested.

36. The use of Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information which is necessary for expression of VP1 and VP2 protein of the human parvovirus B19, in an IFA or ELISA for detecting antibodies directed against the B19 virus in a sample to be tested.

37. A vaccine preparation for inducing an immune response which provides protection against the human parvovirus B19, comprising recombinant virus-like particles consisting of VP1 and VP2 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of these B19 virus proteins, in combination with one or more carriers and/or adjuvants suitable for vaccination purposes.

38. The use of recombinant virus-like particles consisting of VP1 and VP2 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of these B19 virus proteins, for inducing an immune response which provides protection against the human parvovirus B19.

39. Recombinant virus-like particles, comprising VP2 protein of the human parvovirus B19, one or more epitopes of proteins of other pathogens having been incorporated into said VP2 protein, said particles having been formed in Spodoptera

frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of the modified VP2 protein.

40. Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of VP2 protein of the human parvovirus B19, one or more epitopes of proteins of other pathogens having been incorporated into said VP2 proteins.

41. A method of producing virus-like particles, comprising VP2 protein of the human parvovirus B19, one or more epitopes of proteins of other pathogens having been incorporated into said VP2 protein, by culturing Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information which is necessary for expression of the modified VP2 protein.

42. A method according to claim 41, wherein the virus-like particles formed in the cells, comprising VP2 protein of the human parvovirus B19, into which VP2 protein one or more epitopes of proteins of other pathogens have been incorporated, are isolated from the cells.

43. Recombinant baculovirus expression vector, equipped with the genetic information that is necessary for expression in Spodoptera frugiperda cells of VP2 protein of the human parvovirus B19, one or more epitopes of proteins of other pathogens having been incorporated into said VP2 protein.

44. Recombinant baculovirus, equipped with the genetic information that is necessary for expression in Spodoptera frugiperda cells of VP2 protein of the human parvovirus B19, one or more epitopes of proteins of other pathogens having been incorporated into said VP2 protein.

45. The use of virus-like particles, comprising VP2 protein of the human parvovirus B19, one or more epitopes of proteins of other pathogens having been incorporated into said VP2 protein,

said particles having been formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of the modified VP2 protein, in an assay for detecting antibodies directed against the incorporated epitopes in a sample to be tested.

46. The use of Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of VP2 protein of the human parvovirus B19, into which VP2 protein one or more epitopes of proteins of other pathogens have been incorporated, in an assay for detecting antibodies directed against the incorporated epitopes in a sample to be tested.

47. A vaccine preparation, comprising virus-like particles, comprising VP2 protein of the human parvovirus B19, into which VP2 protein one or more epitopes of proteins of other pathogens have been incorporated, which particles have been formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information necessary for expression of the modified VP2 protein, in combination with one or more carriers and/or adjuvants suitable for vaccination purposes, for inducing an immune response which provides protection against these other pathogens.

48. The use of virus-like particles, comprising VP2 protein of the human parvovirus B19, into which VP2 protein one or more epitopes of proteins of other pathogens have been incorporated, which particles have been formed in Spodoptera frugiperda cells which by means of a baculovirus expression vector system have been equipped with the genetic information that is necessary for expression of the modified VP2 protein, for inducing an immune response which provides protection against said pathogens.

INTERNATIONAL SEARCH REPORT

International Application No PCT/NL 90/00130

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 12 N 15/35, C 12 N 5/10, C 12 P 21/02, C 12 N 15/86, G 01 N 33/569, A 61 K 39/23, A 61 K 39/295

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 07 K, C 12 N, A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Bio/Technology, volume 6, no. 1, January 1988 V.A. Luckow et al.: "Trend in the development of baculovirus expression vectors", pages 47-55 see table 1	1-48
Y	Bio/Technology, volume 5, no. 10, October 1987, (New York, US), W.P. Sisk et al.: "Expression of human parvovirus B19 structural protein in E. coli and detection of antiviral antibodies in human serum", pages 1077-1088 see the whole article cited in the application	1-48
P,A	EP, A, 0341611 (BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.) 15 November 1989 see the whole document	1-48

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14th December 1990	24 JAN 1991
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. NL 9000130 SA 40044

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 16/01/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0341611	15-11-89	None	

INTERNATIONAL APPLICATION UNDER THE PATENT COOPERATION TREATY

REQUEST

THE UNDERSIGNED REQUESTS THAT THE PRESENT INTERNATIONAL APPLICATION BE PROCESSED ACCORDING TO THE PATENT COOPERATION TREATY

(The following is to be filled in by the receiving Office:)
INTERNATIONAL APPLICATION No:

INTERNATIONAL FILING DATE:

(Stamp)
Name of receiving Office and "PCT International Application"

Applicant's or Agent's File Reference (indicated by applicant if desired) PCT 0172

Box No. I TITLE OF INVENTION

Human parvovirus B19 proteins and virus-like particles, their production, and their use in diagnostic assays and vaccines.

Box No. II APPLICANT (WHETHER OR NOT ALSO INVENTOR); DESIGNATED STATES FOR WHICH HE/SHE/IT IS APPLICANT. Use this box for indicating the applicant or, if there are several applicants, one of them. If more than one person (includes, where applicable, a legal entity) is involved, continue in Box No. III.

The person identified in this box is (check one only): [ ] applicant and inventor* [X] applicant only

Name *and* address:**

Rijksuniversiteit te Leiden
Stationsweg 46
2312 AV Leiden
the Netherlands

Telephone number (including area code) | Telegraphic address: | Teleprinter address:

Country of nationality: NL | Country of residence:*** NL

The person identified in this box is *applicant* for the purposes of (check one only):

[ ] all designated States [X] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the "Supplemental Box"

Box No. III FURTHER APPLICANTS, IF ANY; (FURTHER) INVENTORS, IF ANY; DESIGNATED STATES FOR WHICH THEY ARE APPLICANTS (IF APPLICABLE). A separate sub-box has to be filled in in respect of each person (includes, where applicable, a legal entity). If the following two sub-boxes are insufficient, continue in the "Supplemental Box," (giving there for each additional person the same indications as those requested in the following two sub-boxes) or by using a "continuation sheet."

The person identified in this sub-box is (check one only): [X] applicant and inventor* [ ] applicant only [ ] inventor only*

Name *and* address:**

Brown, Caroline Sarah
Frans van Mierisstraat 85 huis
1071 RM Amsterdam
the Netherlands

If the person identified in this sub-box is *applicant (or applicant and inventor)*, indicate also:

Country of nationality: GB | Country of residence:*** NL

and whether that person is *applicant* for the purposes of (check one only):

[ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the "Supplemental Box"

The person identified in this sub-box is (check one only): [ ] applicant and inventor* [ ] applicant only [ ] inventor only*

Name *and* address:**

If the person identified in this sub-box is *applicant (or applicant and inventor)*, indicate also:

Country of nationality: | Country of residence:***

and whether that person is *applicant* for the purposes of (check one only):

[ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the "Supplemental Box"

* If the person indicated as "applicant and inventor" or as "inventor only" is not an *inventor* for the purposes of all the designated States, give the necessary indications in the "Supplemental box."

** Indicate the name of a natural person by giving his/her family name first followed by the given name(s). Indicate the name of a legal entity by its full official designation. In the address, include both the postal code (if any) and the country (name).

*** If residence is not indicated, it will be assumed that the country of residence is the same as the country indicated in the address.

Box No. IV AGENT (IF ANY) OR COMMON REPRESENTATIVE (IF ANY); ADDRESS FOR NOTIFICATIONS (IN CERTAIN CASES). A common representative may be appointed only if there are several applicants and if no agent is or has been appointed; the common representative must be one of the applicants.
The following person (includes, where applicable, a legal entity) is hereby/has been appointed as agent or common representative to act on behalf of the applicant(s) before the competent International Authorities:

Name *and* address, including postal code and country:

Smulders, Th.A.H.J.
c/o VEREENIGDE OCTROOIBUREAUX
Nieuwe Parklaan 107
2587 BP The Hague
the Netherlands

If the space below is used instead for an address for notifications, mark here ☐

Telephone number (including area code): 070-3500464
Telegraphic address:
Telex: 32270 union nl
Teleprinter address: 070-3522723

Box No. V DESIGNATION OF GROUPS OF STATES OR STATES (1); CHOICE OF CERTAIN KINDS OF PROTECTION OR TREATMENT. The following designations are hereby made (please mark the applicable check-boxes):

Regional Patent

[X] EP **European Patent**(2): AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany (Federal Republic of), DK Denmark, ES Spain, FR France, GB United Kingdom, IT Italy, LU Luxembourg, NL Netherlands, SE Sweden,
and any other State which is a Contracting State of the European Patent Convention and of the PCT

☐ OA **OAPI Patent**: Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Congo, Gabon, Mali, Mauritania, Senegal, Togo,
and any other State which is a Contracting State of OAPI and of the PCT; if other OAPI title desired, specify on dotted line(3): .....

National Patent (if other kind of protection or treatment desired, specify on dotted line(3))

☐ AT Austria(3) .....
☐ AU Australia(3) .....
☐ BB Barbados
☐ BG Bulgaria(3) .....
☐ BR Brazil(3) .....
☐ CA Canada
☐ CH and LI Switzerland and Liechtenstein
☐ DE Germany (Federal Republic of)(3) .....
.....
☐ DK Denmark
☐ ES Spain(3) .....
☐ FI Finland
☐ GB United Kingdom
☐ HU Hungary
☐ JP Japan(3) .....
☐ KP Democratic People's Republic of Korea(3) .....
.....
☐ KR Republic of Korea(3) .....
☐ LK Sri Lanka
☐ LU Luxembourg(3) .....
☐ MC Monaco(3) .....
☐ MG Madagascar
☐ MW Malawi(3) .....
☐ NL Netherlands
☐ NO Norway
☐ RO Romania
☐ SD Sudan
☐ SE Sweden
☐ SU Soviet Union(3) .....
.....
[X] US United States of America(3) .....
.....

Space reserved for designating States (for the purposes of a national patent) which have become party to the PCT after the issuance of this sheet:

.....

(1) The applicant's choice of the order of designations may be indicated by marking the check-boxes with sequential arabic numerals (see also the "Notes to Box No. V").
(2) The selection of particular States for a European patent can be made upon entering the national (regional) phase before the European Patent Office (see also the "Notes to Box No. V").
(3) If another kind of protection or a title of addition or, in the United States of America, treatment as a continuation or a continuation-in-part is desired, specify according to the instructions given in the "Notes to Box No. V."

See notes on accompanying sheet

Box No. VI PRIORITY CLAIM (IF ANY). The priority of the following earlier application(s) is hereby claimed.

	Country (country in which it was filed if national application; one of the countries for which it was filed if regional or international application)	Filing Date (day, month, year)	Application No.	Office of Filing (fill in only if the earlier application is an international application or a regional application)
(1)	NL	14 september 1989 14. 09. 89	8902301	
(2)				
(3)				

(Letter codes may be used to indicate country and/or Office of filing)

When the earlier application was filed with the Office which, for the purposes of the present international application, is the receiving Office, the applicant may, *against payment of the required fee*, ask the following:

☐ the receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the above-mentioned earlier application/of the earlier applications identified above by the numbers (insert the applicable numbers)

Box No. VII EARLIER SEARCH (IF ANY). Fill in where a search (international, international-type or other) by the International Searching Authority has already been requested (or completed) and the said Authority is now requested to base the international search, to the extent possible, on the results of the said earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.

International application number or number and country (or regional Office) of other application: 8902301

International/regional/national filing date: 14. 09. 89
14 september 1989

Date of request for search: 24. 01. 90
24 january 1990

Number (if available) given to search request: SN 14954 NL

Box No. VIII SIGNATURE OF APPLICANT(S) OR AGENT

J. H. A. Doeve

If the present Request form is signed on behalf of any applicant by an agent, a separate power of attorney appointing the agent and signed by the applicant is required. If in such case it is desired to make use of a general power of attorney (deposited with the receiving Office), a copy thereof must be attached to this form.

Box No.IX CHECK LIST (To be filled in by the Applicant)

This international application contains the following number of sheets:

1.	request	3	sheets
2.	description	18	sheets
3.	claims	8	sheets
4.	abstract	1	sheets
5.	drawings	1	sheets
	Total	31	sheets

Figure number 1 of the drawings (if any) is suggested to accompany the abstract for publication.

This international application as filed is accompanied by the items checked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ priority document(s) (see Box No. VI)
4. ☐ receipt of the fees paid or revenue stamps
5. ☐ cheque for the payment of fees
6. ☒ request to charge deposit account
7. ☒ other document (specify) "Fee Ca:culation Sheet"

(The following is to be filled in by the receiving Office)

1. Date of actual receipt of the purported international application:
2. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
3. Date of timely receipt of the required corrections under Article 11 of the PCT:
4. Drawings ☐ Received ☐ No Drawings

(The following is to be filled in by the International Bureau)

Date of receipt of the record copy: